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Modifications of a large HIV prevention clinical trial to fit changing realities: A case study of the Breastfeeding, Antiretroviral, and Nutrition (BAN) Protocol in Lilongwe, Malawi

Charles van der Horst, MD[1], **Charles Chasela, MPH**[2], **Yusuf Ahmed, BM**[3], **Irving Hoffman, PA, MPH**[1], **Mina Hosseinipour, MD, MPH**[1], **Rodney Knight, PhD**[9], **Susan Fiscus, PhD**[1], **Michael Hudgens, PhD**[1], **Peter Kazembe, MBChB**[4,5], **Margaret Bentley, PhD**[1], **Linda Adair, PhD**[1], **Ellen Piwoz, PhD**[6], **Francis Martinson, MBChB, PhD**[2], **Ann Duerr, MD, PhD**[7], **Athena Kourtis, MD, PhD**[3], **A. Edde Loeliger, MD, MSc**[8], **Beth Tohill, PhD**[3], **Sascha Ellington, MSPH**[3], **Denise Jamieson, MD, MPH**[3], and **for the BAN Study team**

[1] University of North Carolina at Chapel Hill, North Carolina, USA

[2] UNC Project, Lilongwe, Malawi

[3] Division of Reproductive Health, Centers for Disease Control and Prevention, Atlanta, Georgia, USA

[4] Kamuzu Central Hospital, Lilongwe, Malawi

[5] Baylor College of Medicine Children's Foundation, Lilongwe, Malawi

[6] Bill & Melinda Gates Foundation, Seattle, Washington, USA

[7] University of Washington, School of Public Health and Community Medicine, Seattle, Washington, USA

[8] GlaxoSmithKline, Greenford, United Kingdom

[9] Principia Inc, Chapel Hill, North Carolina, USA

*Reprint Requests should be addressed to: Charles van der Horst, MD, Center for Infectious Diseases, 130 Mason Farm Road, Second Floor, CB# 3368, Chapel Hill, NC 27599-3368, Tel.: +1-919-843-4375 Fax: +1-919-966-8536, cvdh@med.unc.edu.

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BAN Study Team at University of North Carolina Chapel Hill, Centers for Disease Control and Prevention, Atlanta, and UNC Project team in Lilongwe including: Linda Adair, Yusuf Ahmed, Mounir Ait-Khaled, Sandra Albrecht, Kant Bangdiwala, Ronald Bayer, Margaret Bentley, Brian Bramson, Emily Bobrow, Nicola Boyle, Sal Butera, Charles Chasela, Charity Chavula, Joseph Chimerang'ambe, Maggie Chigwenembe, Maria Chikasema, Norah Chikhungu, David Chilongozi, Grace Chiudzu, Lenesi Chome, Anne Cole, Amanda Corbett, Amy Corneli, Ann Duerr, Henry Eliya, Sascha Ellington, Joseph Eron, Sherry Farr, Yvonne Owens Ferguson, Susan Fiscus, Shannon Galvin, Laura Guay, Chad Heilig, Irving Hoffman, Elizabeth Hooten, Mina Hosseinipour, Michael Hudgens, Stacy Hurst, Lisa Hyde, Denise Jamieson, George Joaki (deceased), David Jones, Zebrone Kacheche, Esmie Kamanga, Gift Kamanga, Coxcilly Kampani, Portia Kamthunzi, Deborah Kamwendo, Cecilia Kanyama, Angela Kashuba, Damson Kathyola, Dumbani Kayira, Peter Kazembe, Rodney Knight, Athena Kourtis, Robert Krysiak, Jacob Kumwenda, Edde Loeliger, Misheck Luhanga, Victor Madhlopa, Maganizo Majawa, Alice Maida, Cheryl Marcus, Francis Martinson, Chrissie Matika, Douglas Mayers, Isabel Mayuni, Marita McDonough, Joyce Meme, Ceppie Merry, Khama Mita, Chimwemwe Mkomawanthu, Gertrude Mndala, Ibrahim Mndala, Agnes Moses, Albans Msika, Wezi Msungama, Beatrice Mtimuni, Jane Muita, Noel Mumba, Bonface Musis, Charles Mwansambo, Gerald Mwapasa, Jacqueline Nkhoma, Richard Pendame, Ellen Piwoz, Byron Raines, Zane Ramdas, John Rublein, Mairin Ryan, Ian Sanne, Christopher Sellers, Diane Shugars, Dorothy Sichali, Wendy Snowden, Alice Soko, Allison Spensley, Jean-Marc Steens, Gerald Tegha, Martin Tembo, Roshan Thomas, Navdeep Thoofer, Hsiao-Chuan Tien, Beth Tohill, Charles van der Horst, Esther Waalberg, Jeffrey Wiener, Cathy Wilfert, Patricia Wiyo, Innocent Zgambo, Chifundo Zimba. Finally and most especially, all the women and infants that have agreed to participate in the study.

Abstract

In order to evaluate strategies to reduce HIV transmission through breast milk and optimize both maternal and infant health among HIV-infected women and their infants, we designed and implemented a large, randomized clinical trial in Lilongwe, Malawi. The development of protocols for large, randomized clinical trials is a complicated and lengthy process often requiring alterations to the original research design. Many factors lead to delays and changes, including study site-specific priorities, new scientific information becoming available, the involvement of national and international human subject committees and monitoring boards, and alterations in medical practice and guidance at local, national, and international levels. When planning and implementing a clinical study in a resource-limited setting, additional factors must be taken into account, including local customs and program needs, language and socio-cultural barriers, high background rates of malnutrition and endemic diseases, extreme poverty, lack of personnel, and limited infrastructure. Investigators must be prepared to modify the protocol as necessary in order to ensure participant safety and successful implementation of study procedures. This paper describes the process of designing, implementing, and subsequently modifying the Breastfeeding, Antiretrovirals, and Nutrition, (BAN) study, a large, ongoing, randomized breastfeeding intervention trial of HIV-infected women and their infants conducted at a single site in Lilongwe, Malawi. We highlight some of the successes, challenges, and lessons learned at different stages during the conduct of the trial.

Keywords

Mother-to-child transmission of HIV; breastfeeding; HIV/AIDS; nutrition; study design and management; antiretroviral drugs

Background

Among the estimated 700,000 children infected with HIV in 2003 worldwide, 315,000 were infected through breast-feeding (1). Although some studies suggest that the risk of HIV transmission is higher in the first months of life, others indicate a relatively constant risk of transmission throughout breastfeeding beyond the first month (2–5). Various factors have been associated with increased HIV transmission through breast milk including mastitis, cracked nipples, elevated maternal plasma and breast milk (BM) viral load, low maternal CD4 count and advanced stage of HIV disease, and mixed feeding (giving infants solid foods or other liquids in addition to breastmilk) (5–15).

Despite the risks of HIV transmission, the many advantages of breastfeeding have been well documented. The practice is known to confer nutritional, immunologic, developmental, psychologic, social, and economic benefits including overall lower infant morbidity and mortality. Compared with formula-fed infants, breastfed infants have fewer gastrointestinal and lower respiratory tract infections and are less likely to develop otitis media, necrotizing enterocolitis and other diseases, particularly in the first six months of life (16–24). The greatest benefits accrue to exclusively breastfed infants (infants who only receive breast milk), who are less likely to have diarrheal or respiratory illness, to develop atopic disease, and to become infected with HIV than infants who receive both breast milk and other liquids or solids(15; 18;25–27).

Breastfeeding also provides benefits to the mother including a delay in resumption of ovulation, resulting in increased child spacing (16). In addition to individual health benefits, there are economic and social benefits due to savings from formula purchases (16;28). Producing breast milk of adequate quantity and quality is, however, nutritionally demanding for mothers, particularly for those who have chronic infections. Unless diet during pregnancy and lactation

is adequate, prolonged breastfeeding is likely to lead to maternal nutritional depletion (inadequate nutritional intake compared to metabolic needs) (29–32). Depletion leading to rapid weight loss may place HIV-infected mothers at greater risk of succumbing to opportunistic infections, indirectly increasing their disease progression and risk of death (32; 33).

Thus, HIV-infected women in resource-limited settings are faced with a tragic dilemma – breastfeeding their infants with its associated risk of HIV transmission or protecting their children from HIV by replacement feeding (anything other than breast milk), which may also be nutritionally less demanding for themselves, but increases the infant's risk of malnutrition and death if replacement feeding is not affordable, feasible, and safe (34;35).

Current WHO recommendations emphasize that (1) breastfeeding should be supported and promoted in the general population, irrespective of HIV infection rates; (2) there should be improved access to HIV counseling and testing; and, (3) HIV-infected women should be fully counseled about the benefits of breastfeeding, the risk of HIV transmission through breastfeeding, and the risks and possible advantages associated with other methods of infant feeding. Exclusive breastfeeding for the first 6 months is recommended in the general population and for HIV positive mothers who breastfeed. Recently revised recommendations state that early breastfeeding cessation should only be considered if criteria for replacement feeding are met (36).

In Malawi, liquid formula is impractical due to the weight and shipping costs. The cost of dry formula is $13.60 month for an infant age 7–12 months, which far exceeds the per capita health budget in most sub-Saharan countries (28). Powdered formula must be mixed with water, which is often contaminated and can lead to severe and sometimes fatal diarrheal disease (17;18; 34). In addition Malawi guidelines follow the WHO guidelines.

In light of the complex risk-benefit ratio of breastfeeding for HIV-infected mothers and their infants, there is an urgent need to identify ways to make breastfeeding safer for both mothers and infants in resource-limited settings. In Lilongwe, Malawi, despite broad coverage of prevention of mother-to-child transmission (PMTCT) programs that offer short-course perinatal antiretroviral regimens to HIV-infected women (37), the risks of HIV transmission during the postnatal period are still substantial due to nearly 100% breastfeeding, almost no weaning before 12 months, and negligible exclusive breastfeeding, thus minimizing the effect of the PMTCT program.

In April 2001 the Centers for Disease Control and Prevention and the University of North Carolina began designing the Breastfeeding, Antiretrovirals and Nutrition Study, hereafter referred to as BAN, a randomized clinical trial to address these issues. The purpose of the study was to evaluate the following: 1) the benefit and safety of antiretroviral prophylaxis given either to infants or to their mothers to prevent HIV transmission during breastfeeding, 2) the benefit of nutritional supplementation given to the women during breastfeeding to prevent maternal depletion, and 3) the feasibility of exclusive breastfeeding followed by early, rapid breastfeeding cessation. To evaluate the first two objectives, two interventions were assessed in a factorial design: 1) a 3-arm postnatal antiretroviral intervention with additional drugs given to the mother or infant, or nothing in addition to an enhanced standard perinatal prophylaxis, and 2) a 2-arm maternal nutritional intervention to promote maternal health with a food supplement given to half the mothers. The third objective, assessing feasibility of exclusive breastfeeding, is assessed in an observational fashion and does not involve a comparison group. This paper describes the process of designing and implementing this protocol in the field, as well the evolution of this protocol to reflect changes that were implemented to meet the

changing local and international realities over a seven year period. We highlight some of the successes, challenges, and lessons learned at different stages during the conduct of the trial.

Methods

Location and Personnel

Malawi is a country slightly smaller than Pennsylvania in size with a population of 13,571,000, bordered by Zambia, Mozambique and Tanzania in southern Africa. The adult literacy is 64% and the gross national income (GNI) is $170 placing Malawi at 203 out of 209 countries in terms of poverty. Per capita annual health expenditure is 58 dollars. Lilongwe is the capital with a population of 744,000. Most of the population speaks Chichewa although most professionals speak English as well. Faculty from the University of North Carolina has been working in Malawi since 1990. In-country activities in Lilongwe are coordinated and overseen through the UNC Project, a program providing clinical care, training, technical advice and research and which is headed by a resident country director and staffed with more than 300 medical and laboratory personnel from Chapel Hill and Malawi including physicians, clinical officers, nurses and nutritionists All of our Malawi and US team members receive training and certification in human subjects protections as well as either Good Clinical or Good Laboratory Practice. Key Malawi team members were brought to the US for additional and more specific laboratory training (cell separation, PCR, microbiology assays) as well as the data management systems we used (Laboratory Data Management System by Frontier Science Foundation and TELEform™ software by Cardiff). We provide health benefits and transportation for staff.

Recruitment and Screening

The University of North Carolina at Chapel Hill currently administers a prevention of mother-to-child transmission (PMTCT) program at four government clinics in Lilongwe, the capital of Malawi, and also provides technical assistance for nine other PMTCT sites run by the Malawi Ministry of Health (36;37). The PMTCT program offers HIV counseling and testing to all pregnant women as well as enhanced antenatal care and single-dose nevirapine prophylaxis given to all HIV-infected women in labor and to their infants shortly after delivery (i.e. the HIVNET 012 regimen). HIV testing is conducted using two simultaneous rapid tests, Abbott Determine HIV-1/2 and Uni-Gold HIV (Trinity Biotech plc, Bray, Eire). The Bioline HIV-1/2 3.0 (Standard Diagnostics, Kyonggi-do, South Korea) is used as a tie-breaker in the event of conflicting results. Among the more than 28,000 pregnant women tested each year, the HIV seroprevalence rate is about 13%. Through this program all HIV positive women receive cotrimoxazole if their CD4 count is less than 500 cells/μL and all HIV-exposed infants receive the same once they reach 6 weeks of age. Antenatally, all women receive iron and folate, screening for syphilis and anemia, malaria prophylaxis, mosquito nets and tetanus toxoid. Postnatally, all infants receive routine vaccinations which include BCG, polio, diphtheria, pertussis, tetanus, Haemophilus influenza, hepatitis B, and measles. All women with low CD4 counts (<200–250 cells/μL), identified either through the PMTCT program or subsequently through the BAN screening process, are referred to the Lighthouse Clinic, a Ministry of Health sponsored facility which provides antiretroviral therapy through the Global Fund.

Women identified as HIV-infected are offered referral to the BAN study clinic at Bwaila Hospital. Women who are willing to participate and intend to breastfeed are asked to sign a screening consent. For women who consent to be screened, specimens are collected and a medical history obtained to assess eligibility. Primary eligibility criteria include: 1) ≤30 weeks gestation, 2) at least 18 years of age (or 14 years of age if married), 3) hemoglobin >7 g/dL, 4) CD4 count ≥ 250 cells/μL, 5) no prior antiretroviral medication use, 6) normal liver function tests (<2.5 upper limit of normal), 7) no serious complications of pregnancy, and 8) not

previously enrolled in the BAN study. Women meeting these eligibility criteria are asked to return for 4 additional antenatal study visits.

All women enrolled in BAN are asked to deliver at Bwaila Hospital. However, women who deliver elsewhere are eligible as long as the mother and infant arrive at Bwaila Hospital for evaluation within 36 hours of birth. In order to be eligible for randomization, additional criteria must be met: 1) infant birth weight ≥2000 grams, 2) no severe congenital malformations or other conditions incompatible with life, and 3) based on clinical assessment, no maternal condition which would preclude start of study drug. Eligible mother-infant pairs are randomized to one of six treatment arms (see section on Factorial Design, and Table 1). Infants found to be perinatally HIV-infected at birth or at two weeks of life, and their mothers, are subsequently disenrolled from the study and referred for recommended antiretroviral treatment.

We collected maternal breastmilk, peripheral blood mononuclear cells (PBMCs), plasma, serum and cell pellets as well as infant PBMCs, plasma, serum, dried blood spots and cell pellets. The specimens were collected for viral loads and genotypes, drug levels, measurement of other viruses, vitamin levels, immunological studies and possible genetic typing.

Antiretroviral, nutritional, and other interventions for all participants

All mother-infant pairs enrolled in the study receive certain antiretroviral medications and nutritional and other benefits, regardless of the arm to which they are assigned. In addition, mother-infant pairs receive medical care from the study staff that exceeds the standard of care for Malawi. For equity purposes and due to seasonal food shortages, all participants received 2 kg of maize each week. Although the nutritive quality of the maize varies over time, this ration provides approximately 200 kcal per person per day for a family of five. Any infant or mother presenting with an intercurrent illness has full medical care provided by UNC Project. To achieve this level of care, we built microbiology and other clinical laboratories for diagnosis and treatment of pneumonia, sepsis, meningitis, infectious diarrhea, tuberculosis, and malaria. All enrolled mothers receive single dose nevirapine (the HIVNET 012 regimen) peripartum plus twice a day zidovudine (ZDV) 300 mg and lamivudine (3TC) 150 mg during labor and for 7 days postpartum. When infants receive their single dose nevirapine (NVP) 2mg/kg after delivery they also begin ZDV (12 mg) plus 3TC (6 mg) twice daily for 7 days. Women receive a single vitamin A supplement post partum and counseling on exclusive breastfeeding (EBF) and on rapid, early breastfeeding cessation between 24 and 28 weeks postpartum. Study subjects receive the equivalent in Malawi currency of US $4 per study visit or interim visits for illness and US $10 for delivering at the health center (see section below for study visit schedule).

Factorial Study Design

After delivery, participants are randomized according to 1 of the 6 treatment conditions using a permuted block method (Table 1). That is, assignments are randomly allocated in groups of 6 or more, in order to ensure a balanced allocation. The group size varies randomly between 6, 12, and 18, in order to prevent discovery of the allocation scheme by study researchers. The study's target sample size is 2418 mother-infant pairs, with 403 assigned to each treatment combination. There are 17 scheduled study visits: 2 antepartum screening visits, an enrollment visit soon after delivery, and postpartum visits at 1, 2, 4, 6, 8, 12, 18, 21, 24, 28, 32, 36, 42, and 48 weeks postpartum. The enrollment visit, during which women are randomized, is generally conducted during the delivery hospitalization unless the woman delivers at another hospital. In addition to the regularly scheduled visits, women who register for the study early may have additional study visits antenatally at 28, 32, and 36 weeks estimated gestational age, as well as postnatally, whenever they feel the need for additional medical care. Data are systematically collected for all unscheduled interim visits.

In this factorial design, half of the study mothers are randomized to receive a high-energy, high-protein, micronutrient-fortified food supplement (Table 2). The supplement provides the daily energy required to support exclusive breastfeeding and 100% of the recommended dietary allowance for all micronutrients except vitamin A, which has been associated with increased postnatal HIV transmission when consumed daily (38). The supplement is supplied for 28 weeks after delivery, or until reported breastfeeding cessation, whichever occurs first.

All women who experience excessive weight loss, defined as 5% or more of body weight, between visits beginning 4 weeks after delivery or a body mass index (BMI=kg/m^2) that falls below 17, are examined by a clinician and provided with nutritional supplement if they were not already receiving it through week 28.

For mothers or infants randomized to the antiretroviral (ARV) arms, study drugs are supplied for 28 weeks after delivery or until reported breastfeeding cessation, if earlier. Infants assigned to the ARV arms receive daily NVP, with doses ranging from 6 mg to 26 mg, increasing as the infant ages up to 28 weeks (39) We utilized a simplified dosing regimen that is easy to administer (Table 3) in a resource-limited setting, as it does not rely on infant weighing. Mothers assigned to the maternal HAART regimen received combination therapy with three drugs. At the beginning of the study, postpartum mothers assigned to HAART received 300 mg ZDV, 150 mg 3TC taken orally every 12 hours for 28 weeks as well as NVP 200 mg once daily for 14 days and then 200 mg every 12 hours. Mothers who develop toxicity to the ZDV component were switched to stavudine and those that are intolerant of NVP were switched to nelfinavir.

The median duration of breastfeeding in Sub-Saharan Africa is 18 months (40). To minimize the risks of malnutrition following early breastfeeding cessation, the study provides mothers with a locally produced ready- to-use therapeutic food (RUTF) commonly used in Malawi for treatment of severe acute malnutrition in children. The RUTF is made from full-cream powdered milk, peanut butter, sugar, oil, and fortified with micronutrients. No water is needed for mixing, making it safer than other products that require reconstitution with potentially contaminated water supplies. Infants can suck the peanut butter spread from a spoon. The breast milk replacement food ration provides approximately 400 kcal and 9.5 g of protein per day for the study children from 28 to 48 weeks of age. Although this RUTF has been used extensively in several countries, (including Malawi, Ethiopia, Chad and Niger) for home treatment of severe acute malnutrition, this study uses it as a breast milk replacement food (41–45). Mothers were also counseled to provide local, complementary weaning foods.

Statistical Analysis

BAN has three primary study outcomes: 1) HIV transmission to infants, 2) maternal depletion, and 3) feasibility of exclusive breastfeeding followed by rapid weaning.

1. Prevention of HIV transmission to infants. Assessment of antiretroviral treatment efficacy is based on differences in the proportion of infants free of HIV infection at 28 weeks. The target sample size of 2418 mother-infant pairs was calculated assuming 7% cumulative HIV infection rate in the control arm by 28 weeks of age in infants who are uninfected at birth or 2 weeks postpartum. This estimate is based on studies of transmission of HIV to the infant through breastfeeding, including a meta-analysis of published evidence (46;47). We allowed for 8% of infants to be disenrolled due to evidence of HIV by 2 weeks (48), an additional 10% of mother-infant pairs to be lost to follow-up by 28 weeks, and 7 interim analyses based on O'Brien-Fleming guidelines. The assumption for the cumulative infant HIV infection rate by 28 weeks in the antiretroviral treatment arms was 3%. Using a 0.025 significance level to adjust for multiple comparisons with the control arm, the sample size was chosen to provide

at least 80% power to reject the null hypothesis of no difference in HIV incidence between the control arm and a treatment arm. Two-sided log-rank tests will test the two null hypotheses comparing (i) maternal HAART versus no maternal or infant antiretrovirals and (ii) infant antiretrovirals versus no maternal or infant antiretrovirals. Infant deaths prior to HIV diagnosis will be analyzed both as right censored observations and as a component of the composite endpoint of death or HIV infection, whichever occurs first. To guard against potential selection bias induced by conditioning on the post-randomization event of HIV infection at 2 weeks we will also conduct an intent-to-treat (ITT) analysis of HIV infection rates whereby all infants will be included in the analysis, regardless of HIV status at birth or 2 weeks. Infant HIV infection is determined by qualitative Roche HIV-1 Amplicor DNA 1.5 PCR at weeks 12 and 28 with requirement for a confirmatory test.

2. Prevention of maternal nutritional depletion. Evaluation of the effectiveness of the nutritional supplement for preventing maternal depletion will be based on weight loss between 4 and 28 weeks post-partum. For this outcome we anticipate that mothers not receiving a supplement will lose 0.17 kg/month and mothers receiving a supplement will lose 0 kg/month with a constant standard deviation 1.32 (32). The study sample size as calculated above has over 90% power to detect a difference of 0.20 kg in mean maternal weight loss from 4 to 28 weeks.

3. Feasibility of exclusive breastfeeding for 24 weeks followed by rapid weaning. We will examine the proportion of mothers reporting breastfeeding cessation and the proportion of infants whose weight for age is below the World Health Organization 5^{th} percentile before and after weaning and infant survival from reported breastfeeding cessation or 28 weeks, whichever is earlier, through 48 weeks. We will also examine growth faltering of infants, defined as a decline of weight for age z-score of more than one standard deviation, as well as HIV transmissions to the infant that occur beyond 28 weeks. Evaluation of this objective will be based on the descriptive data mentioned above rather than quantitative *a priori* assumptions.

Results

The initial study idea was created in response to an April 2001 Request for Proposals from the US Centers for Disease Control and Prevention, submitted in June 2001 and funded in October 2001. As the protocol was being developed, we conducted a series of formative research projects over a two-year period in Lilongwe. Using data generated from multiple focus groups, client home visits, and individual in-depth interviews and taste tests of the nutritional supplement among pregnant women, spouses, civic leaders, healthcare workers, HIV-infected breastfeeding women and family members, the team gauged the feasibility of the proposed protocol (49;50). This shaped many decisions in protocol design; for example, we learned that mothers considered drawing blood from healthy babies at birth feasible and acceptable but had concerns about the volume to be drawn, and we used this information when formulating the biological sample schedule and volumes. We also learned that mothers preferred a longer weaning period, rather than more rapid weaning, for their own and the babies' comfort and safety and to assure good infant transition to other foods. This shaped our decision to allow a month-long weaning period at 6 months of infant age. Mothers expressed concern about the impact of weaning on the nutritional status of their infants and so we provide locally produced RUTF from weaning through 48 weeks post-partum. We also tested the acceptability among mothers of the choice of the maternal nutrition supplement. We placed emphasis on mother's health during breastfeeding, and provided a family food supplement. Without the formative research many of these issues may have gone unaddressed and could have undermined the successful implementation of the BAN study. The result was an evidence-based protocol

ensuring participant understanding of the research, safeguards for the participants, and increased feasibility and acceptance of clinical research in this community.

While English is the official language of Malawi, Chichewa is the common local language widely used throughout the country. Though many of our patients do not speak English, all study staff spoke both English and Chichewa. The adult literacy rate in Malawi is approximately 64% (51). Prior to initial enrollment, the language of our consent forms was dramatically simplified compared to the standard language found in many standard consent forms. Due to language and literacy issues, we felt that a long and complicated consent form was not ethical. For example, we did not mention chemical names of compounds but rather emphasized the potential toxicities they caused. All consents were translated into Chichewa and then back translated by a separate team into English to ensure that they met international standards. All study forms, which contained questions to be asked patients, were in Chichewa.

The study team used the WHO CIOMS Guidelines for Biomedical Research Involving Human Subjects when the study was designed and held an ethics consultative meeting with an outside ethicist prior to submitting the final protocol.

Once the final protocol was developed, it was submitted for review at three separate institutional review boards in 2003: the parent institution, the sponsoring agency, and the host country. Since each IRB had its own comments on the protocol, we reconciled all with final approval by all three IRBs in March 2004, thirty months after the initial award. In parallel with this process, negotiations for donations of study drugs were conducted.

BAN began enrolling participants in April 2004. Initially, enrollment was relatively slow. In order to increase enrollment, we engaged in community outreach to dispel rumors about the study, including discussions with tribal elders and leaders. We noticed that many women who initially expressed interest in the study failed to return for their screening and enrollment visits. Upon further questioning, it became clear that many husbands were not supportive of the study. To address this, we developed community activities specifically targeting men, such as weekly support groups for men. In addition to community activities, two recruitment sites were added in other areas of Lilongwe. Although the main study activities, including all clinical visits, remained at one site, these two additional sites provided a larger pool of potential study participants. By February 2006, enrollment had increased to our target of 15 enrollees per week. It has remained relatively stable since.

Prior to initiating the study we realized that unlike shorter term studies, we would be following these women and their infants for more than 12 months. Usually, such care is provided by the understaffed public sector, which also suffers from frequent stock outs of critical medicines and often lack diagnostic facilities. Since we felt an ethical obligation to provide clinical care for intercurrrent illnesses, we purchased all the medications for intercurrent illnesses, hired additional staff to provide for non-study related care and built a microbiology laboratory. Although we have not completed a formal cost analysis such clinical care consumes an additional 25% of the study costs.

To date, there have been twelve subsequent amendments made to the protocol and submitted to the three regulatory agencies (Table 4). Modifications were made to increase toxicity monitoring in light of new data on nevirapine hepatotoxicity (52), ours and others' discovery of benign ethnic infant neutropenia (53;54) which is a temporary, clinically insignificant laboratory finding, better understanding of nevirapine resistance (55), FDA advisories (56), changing national and international guidelines for treatment of adults and need for cotrimoxazole prophylaxis (57;58), a change in the NIH Toxicity Tables, and several changes to decrease the protocol burden on the clients and the staff. We also decided that longer follow up may be necessary and thus altered the consent form to allow for contact after the study

ended. These amendments can be grouped as those implemented to address toxicities, those to decrease the enormous workload of the study, and those to meet the changing reality of HIV care. After the initial IRB application, which took many months of negotiating between the three IRBs, the process has decreased to 2 months or less for each amendment. Although, each of the IRBs would sometimes ask for clarification or ask us to amend the consent form, there were no excessive delays or unwanted requests. On two occasions, for safety and ethical reasons the IRB's were notified simultaneously of the problem and our action plan. This applied to our handling of the nevirapine toxicity issue and after the DSMB recommendation to stop one arm.

Amendments for new information regarding toxicities

Within weeks of assigning women to HAART containing NVP, we became aware of a need to increase the monitoring for hepatic toxicity. In February 2004, Boehringer-Ingelheim had sent a "Dear Doctor" letter outlining changes to the Black Box Warning for nevirapine, which stated that women with CD4 counts greater than 250 cells/μL had a 12-fold higher risk of hepatotoxicity, usually within six weeks of initiating therapy. On January 19, 2005 the FDA strengthened the warning to recommend against starting nevirapine treatment in women with CD4 counts greater than 250 cells/μL unless benefits clearly outweigh risk (56). Among the first 39 women randomized who received nevirapine-containing HAART, we found 3 severe rashes, 1 case of clinical hepatitis and 5 cases of severe elevations in hepatic transaminases. We subsequently have shown that nevirapine drug levels are higher in Africans compared to Americans (59). Even though we did not have any fatal complications, conducting a study in a resource-limited country requires a higher standard beyond safety and efficacy; that of feasibility of eventual national roll out of the regimen. The requirement for close monitoring of transaminases necessitating a large number of clinical and laboratory staff clearly made this an infeasible regimen for eventual implementation in breastfeeding women with high CD4 counts in Malawi. We initially switched all breastfeeding mothers assigned to HAART from nevirapine to our second-line regimen containing nelfinavir and ultimately, for reasons of availability, safety and potency, to lopinavir/ritonavir (Kaletra).

The NIH toxicity tables were developed by the NIH Division of AIDS (DAIDS) in the beginning of the AIDS epidemic in the United States. Although widely used in clinical trials, we found early in the conduct of BAN that they do not take into account the benign neonatal neutropenia seen in both African-Americans and black Africans (53;54). In our first months we noticed that of 206 newborns enrolled, 22 had grade 3 or 4 neutropenia (9 present at birth) without any clinical sequelae and with spontaneous recovery of the absolute neutrophil counts within weeks. We, therefore, modified the infant neutropenia criteria for our protocol to define grade three toxicity for any age infant to include an ANC less than 400 cells/μL, which decreased the incidence of grade 3/4 neutropenia to less than 1%. Subsequently, DAIDS did modify their toxicity table, although not to the extent of our revision (Table 5).

In 2006 the NIH Division of AIDS modified infant anemia criteria to state that any hemoglobin less than 9.0 g/dl for HIV negative infants at least 57 days old was at least a grade 3 toxicity. The previous version cited a hemoglobin value of less than 7 g/dl as the cut-point for grade 3 anemia. This new table was adopted by BAN in May 2006 and led to an artificial increase in grade 3/4 anemia from 55 newborn infants to 238 in the first 1233 mother infant pairs randomized.

Amendments to decrease workload

With a weekly accrual target of 15 mother-infant pairs, the overall workload of BAN is considerable. Due to the complex design and multiple objectives, lengthy questionnaires, and the need for close participant monitoring, study visits were taking up to 8 hours to complete, sometimes requiring drivers to take clients home in the evening, in some cases requiring return

visits, and placing an undue burden on participants. In addition, the sheer volume of data generated, including over 20,000 pages scanned per month, jeopardized the quality of the data obtained. With the onset of the rainy season each November in Lilongwe, sick (i.e. unscheduled) visits to the study clinic increased precipitously, placing a huge burden on project staff and increasing study costs dramatically. From July 2005 until June 2006, there were 1800 scheduled study visits and 400 unscheduled visits for the first 883 mother infant pairs randomized, usually due to intercurrent illnesses. These additional visits increased annual costs by $362,000 (29%) in additional personnel and laboratory costs.

With this burden on clients, staff and study budget, we quickly realized that several elements of the original protocol were not feasible. Since many of our patients are not literate, our staff would read the consent to them, pausing for questions and clarifications. In addition, the study staff uses a consenting checklist to ensure that important areas have been covered and are understood by the client. The study consent process was taking up to 2 hours to complete. Since 28% of consented women were subsequently determined to be ineligible, (half because they did not meet primary eligibility due to CD4 count or hemoglobin level and half because they delivered at home and did not present in time to the study clinic or had other complications), we decided to split the consent process into a screening registration form and a post-partum randomization form. This both shortened the original consent and the time imposed on the patients as well as sparing those, who would not be eligible, from having to sit through the description of the study after delivery. In addition, the PMTCT program, which refers potential participants to BAN, began routine CD4 testing thereby allowing women with low CD4 counts to be excluded from the study and referred to appropriate care without referral to BAN.

The burden on study participants was also great due to the number and length of visits. In order to complete the study, mothers came to clinic for at least 17 visits and infants for at least 15 visits with blood drawn at every visit. When 900 women-infant pairs had been randomized, we systematically reduced the data collected to focus on key study endpoints. Case report forms across the study had questions reduced. Reductions were made in demographic, delivery history, breastfeeding, infant feeding, infant and maternal anthropometry, ARV adherence, lab and physical exam forms. Twenty of the over 70 forms in the study were modified. After we had randomized 1600 mother infant pairs, we further reduced the case report forms. This reduction was mainly through the elimination of forms or number of times a form, such as the infant and maternal 24-hour dietary recall, is administered. Deleted forms included the breast health history, detailed breast exam, and detailed information about breastfeeding and early weaning practices. At the peak, we estimate that collectively, staff was spending 1941 hours per month filling out forms, which was reduced to an average of 1608 hours after the first reduction in 2006 and to 1135 hours after the second reduction in 2007, resulting in an overall reduction in workload of approximately 40%. Although we had pilot tested many of the forms, in retrospect we realized that in advance of the study it would have been helpful to devote more time to practice runs of the visit procedures including completion of the forms.

We originally planned to use case report forms with diagnostic algorithms for each opportunistic and endemic infection similar to those used in US HIV clinical trials. Even with modifications reflecting locally available tests, it was clear that these forms were infeasible. For instance, almost no one obtained a chest radiograph for initial diagnosis of pneumonia. We stopped using those forms and substituted an adverse events log to make it easier for staff to track onset and completion of various events including adverse events, endemic and opportunistic infections and concomitant medications.

In addition to reducing data collection, we also cut back on specimen collection. Thirty-nine months into the study we stopped freezing cell pellets, peripheral blood mononuclear cells, and pharmacokinetic samples in order to cut costs. Previously, we were freezing on average

5,680 breast milk vials, 3,100 plasma vials, 500 cell pellet vials and 1,400 peripheral blood mononuclear cell vials monthly. After implementing our amendments this decreased to 1,350 plasma vials monthly. None of the primary endpoints required any of the discontinued questions or specimens.

Amendments to meet the changing realities of HIV care

In a study initiated during 2004 in a rapidly changing field, it was unavoidable that new data would appear that might require changes to the protocol. In an effort to minimize development of nevirapine resistance in women randomized to a HAART regimen containing nevirapine, we added a 7-day Combivir tail after the nevirapine was stopped. This was an approximate adjustment for the prolonged serum half-life of nevirapine compared to the relatively short half-life of Combivir.

The Malawi Ministry of Health guidelines for HAART treatment changed the CD4 threshold below which antiretroviral therapy was to be started from a CD4 count of <200 cell/ μL to < 250 cell/μL. In response, we changed the BAN enrollment criteria to exclude women who had CDC counts of <250 cell/ μL; these women were referred for antiretroviral therapy. The Malawi Ministry of Health also instituted routine cotrimoxazole prophylaxis for HIV-infected adults and HIV-exposed infants. As of December 2005, all women in BAN with CD4 counts <500 cell/μL were started on cotrimoxazole after the first trimester. In addition, all children aged 6 weeks or above received cotrimoxazole until 3 months after discontinuation of breastfeeding. For HIV-infected infants the prophylaxis is continued indefinitely.

Discussion

With a target sample size of 4836 total participants, which includes 2418 mothers and 2418 infants, BAN is among the largest single-site HIV trials in Africa (27;34;47;60–66). To conduct a study of this magnitude, it is critical that the study leadership stay focused on the primary objectives and that components, which place an undue burden on the study staff and the participants are quickly identified and minimized. In addition, large and complex studies like BAN must be flexible enough to rapidly respond to evolving changes in both science and national and international policy, as well as the demands of multiple institutional ethics and monitoring boards. When enrollment takes a long time to complete, results from on-going related studies may affect clinical equipoise between study arms and necessitate alterations to the protocol (67;68). If changes cannot be rapidly implemented, then enrollment may have to be temporarily or permanently suspended, which can disrupt study flow and undermine staff and participant trust and consequently future enrollment. When the information about liver toxicity of nevirapine in women with high CD4 counts emerged, BAN was able to promptly switch the women from nevirapine to the second-line protease inhibitor and inform the IRBs without having to halt enrollment. In addition, preliminary findings from BAN helped shape the conduct of other related studies. For example, the benign ethnic infant neutropenia in African infants that we noted early on in BAN helped support the modifications of the DAIDS toxicity table.

There are several potential disadvantages to protocol modifications once a study has begun. These include inducing confusion in study staff, participants and the wider community. We minimized this by conducting regular staff training around each major protocol change combined with extensive community outreach by a dedicated group of community nurses. Discontinuation of samples and forms collected must always be viewed through the lens of its impact on the major primary and secondary endpoints and to ensure that data quality and the ultimate outcomes do not suffer. Protocol changes occur over time and conditions can change over time as well, which can induce unanticipated bias. These include local endemic diseases,

which vary with the rainy season, such as malaria and diarrheal disease and the nutritional status of the study participants.

By focusing on both maternal and infant outcomes, BAN is unique among mother-to-child HIV transmission trials, which tend to focus primarily on HIV transmission to the infant. In addition to the importance of promoting women's health in its own right, there is evidence that infants born to HIV-infected women, who subsequently die, are at markedly increased risk of morbidity and mortality, even when they themselves are uninfected (69). Therefore, it is imperative that trials balance the risks and benefits of infant feeding options for both the mother and the infant. Another unique component of BAN is the inclusion of both an antiretroviral intervention as well as a nutritional intervention. We chose a factorial design in order to efficiently study two separate research questions evaluating the effectiveness of two independent interventions within the same study population. Use of a factorial design reduced costs, personnel and resources needed to answer two study questions, which is crucial in an area of limited staff and resources, such as Malawi.

Another issue that many large trials face is attracting participants. We did experience initial slow enrollment in BAN which we were able to successfully address by adding two recruitment sites and by actively engaging the community, including local leaders and the women's male partners and dispelling rumors that inevitably arise and are related to the local societal and political context. In this respect, the importance of including social scientists such as anthropologists and health behavior experts from both Malawi and the US as part of the research team and the need for formative research cannot be overemphasized; formative research at the beginning of our trial helped inform the design of several study processes, including consenting study participants, study visit flow, blood sampling, nutritional counseling and broader community engagement. Including local investigators on the design team with an eye to conform the protocol to local culture is essential. Although we did not conduct a pilot study, we believe that had we done this, fewer amendments would have been necessary. Formative research and pilot studies conducted before hand can gauge acceptance of the protocol design, potential pitfalls and solutions to them.

One of the greatest challenges for BAN has been the provision of clinical care in a setting of limited staff and resources. At 79 deaths/1,000 live births (70) and 1100 deaths/100,000 live births (71) respectively, Malawi's infant mortality rate and maternal mortality rate are among the highest in the world. Women's life expectancy is low at 45.7 years (72). Conducting a large randomized trial in such a context of high disease burden and limited resources requires that the study provide a high volume of clinical care and be prepared for a large number of unscheduled visits for illness. If not appropriately factored in when planning the study, this can severely drain study resources, as we learned firsthand throughout the conduct of this study. This is an ethical imperative that funding agencies must address.

Recruiting and training skilled personnel can be difficult. For example, like many parts of the world, there is a severe nursing shortage in Malawi, with many highly trained nurses emigrating to other parts of the world (73). In order to not drain national resources, BAN has a written policy of not hiring nurses currently working for the government. Within this context, hiring and retaining nurses for BAN is challenging. However, the provision of training and other professional opportunities for BAN staff has allowed us to attract and retain many qualified staff members.

The research questions BAN addresses, how to minimize HIV transmission to infants postnatally while ensuring optimal infant nutrition and maximizing maternal health, are multifactorial and complex, thereby requiring a multi-component and complex study design. Our hope is that what we learn from BAN will help shape national policy in Malawi and

international guidance from WHO for how best to feed infants born to HIV-infected women and how best to support lactating HIV-infected women in resource-limited settings where formula feeding is not feasible, safe, and affordable.

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Reference List

1. Coovadia H. Antiretroviral agents--how best to protect infants from HIV and save their mothers from AIDS. N Engl J Med 2004;351(3):289–292. [PubMed: 15247337]
2. Breastfeeding and HIV International Transmission Study Group. Late postnatal transmission of HIV-1 in breast-fed children: an individual patient data meta-analysis. Journal of Infectious Diseases 2004;189(12):2154–2166. [PubMed: 15181561]
3. Miotti PG, Taha TE, Kumwenda NI, et al. HIV transmission through breastfeeding: a study in Malawi. JAMA 1999;282(8):744–749. [PubMed: 10463709]
4. Nduati R, John G, Mbori-Ngacha D, et al. Effect of breastfeeding and formula feeding on transmission of HIV-1: a randomized clinical trial. JAMA 2000;283(9):1167–1174. [PubMed: 10703779]
5. Taha TE, Hoover DR, Kumwenda NI, et al. Late postnatal transmission of HIV-1 and associated factors. J Infect Dis 2007;196(1):10–14. [PubMed: 17538877]
6. Embree JE, Njenga S, Datta P, et al. Risk factors for postnatal mother-child transmission of HIV-1. AIDS 2000;14(16):2535–2541. [PubMed: 11101065]
7. Eshleman SH, Lie Y, Hoover DR, et al. Association between the replication capacity and mother-to-child transmission of HIV-1, in antiretroviral drug-naive Malawian women. J Infect Dis 2006;193(11): 1512–1515. [PubMed: 16652278]
8. Fawzi W, Msamanga G, Spiegelman D, et al. Transmission of HIV-1 through breastfeeding among women in Dar es Salaam, Tanzania. J Acquir Immune Defic Syndr 2002;31(3):331–338. [PubMed: 12439210]
9. John GC, Nduati RW, Mbori-Ngacha DA, et al. Correlates of mother-to-child human immunodeficiency virus type 1 (HIV-1) transmission: association with maternal plasma HIV-1 RNA load, genital HIV-1 DNA shedding, and breast infections. J Infect Dis 2001;183(2):206–212. [PubMed: 11120927]
10. Mwapasa V, Rogerson SJ, Kwiek JJ, et al. Maternal syphilis infection is associated with increased risk of mother-to-child transmission of HIV in Malawi. AIDS 2006;20(14):1869–1877. [PubMed: 16954728]
11. Pillay K, Coutsoudis A, York D, Kuhn L, Coovadia HM. Cell-free virus in breast milk of HIV-1-seropositive women. J Acquir Immune Defic Syndr 2000;24(4):330–336. [PubMed: 11015149]
12. Rousseau CM, Nduati RW, Richardson BA, et al. Longitudinal analysis of human immunodeficiency virus type 1 RNA in breast milk and of its relationship to infant infection and maternal disease. J Infect Dis 2003;187(5):741–747. [PubMed: 12599047]
13. Rousseau CM, Nduati RW, Richardson BA, et al. Association of levels of HIV-1-infected breast milk cells and risk of mother-to-child transmission. J Infect Dis 2004;190(10):1880–1888. [PubMed: 15499546]
14. Semba RD, Kumwenda N, Hoover DR, et al. Human immunodeficiency virus load in breast milk, mastitis, and mother-to-child transmission of human immunodeficiency virus type 1. J Infect Dis 1999;180(1):93–98. [PubMed: 10353866]

15. Coovadia HM, Rollins NC, Bland RM, et al. Mother-to-child transmission of HIV-1 infection during exclusive breastfeeding in the first 6 months of life: an intervention cohort study. Lancet 2007;369 (9567):1107–1116. [PubMed: 17398310]
16. American Academy of Pediatrics. Work Group on Breastfeeding. Breastfeeding and the use of human milk. Pediatrics 1997;100(6):1035–1039. [PubMed: 9411381]
17. WHO Collaborative Study Team on the Role of Breastfeeding on the Prevention of Infant Mortality. Effect of breastfeeding on infant and child mortality due to infectious diseases in less developed countries: a pooled analysis. Lancet 2000;355(9202):451–455. [PubMed: 10841125]
18. Feachem RG, Koblinsky MA. Interventions for the control of diarrhoeal diseases among young children: promotion of breast-feeding. Bull World Health Organ 1984;62(2):271–291. [PubMed: 6610496]
19. Goldman AS. The immune system of human milk: antimicrobial, antiinflammatory and immunomodulating properties. Pediatr Infect Dis J 1993;12(8):664–671. [PubMed: 8414780]
20. Morrow-Tlucak M, Haude RH, Ernhart CB. Breastfeeding and cognitive development in the first 2 years of life. Soc Sci Med 1988;26(6):635–639. [PubMed: 3363405]
21. Newburg DS, Peterson JA, Ruiz-Palacios GM, et al. Role of human-milk lactadherin in protection against symptomatic rotavirus infection. Lancet 1998;351(9110):1160–1164. [PubMed: 9643686]
22. Pabst HF. Immunomodulation by breast-feeding. Pediatr Infect Dis J 1997;16(10):991–995. [PubMed: 9380478]
23. Sirota L, Straussberg R, Notti I, Bessler H. Effect of human colostrum on interleukin-2 production and natural killer cell activity. Arch Dis Child Fetal Neonatal Ed 1995;73(2):F99–102. [PubMed: 7583615]
24. Asha Bai PV, Leela M, Subramaniam VR. Adequacy of breast milk for optimal growth of infants. Trop Geogr Med 1980;32(2):158–162. [PubMed: 7423605]
25. Coutsoudis A. Promotion of exclusive breastfeeding in the face of the HIV pandemic. Lancet 2000;356 (9242):1620–1621. [PubMed: 11089813]
26. Coutsoudis A, Pillay K, Kuhn L, Spooner E, Tsai WY, Coovadia HM. Method of feeding and transmission of HIV-1 from mothers to children by 15 months of age: prospective cohort study from Durban, South Africa. AIDS 2001;15(3):379–387. [PubMed: 11273218]
27. Iliff PJ, Piwoz EG, Tavengwa NV, et al. Early exclusive breastfeeding reduces the risk of postnatal HIV-1 transmission and increases HIV-free survival. AIDS 2005;19(7):699–708. [PubMed: 15821396]
28. Maclean CC, Stringer JS. Potential cost-effectiveness of maternal and infant antiretroviral interventions to prevent mother-to-child transmission during breast-feeding. J Acquir Immune Defic Syndr 2005;38(5):570–577. [PubMed: 15793368]
29. Adair LS, Popkin BM. Prolonged lactation contributes to depletion of maternal energy reserves in Filipino women. J Nutr 1992;122(8):1643–1655. [PubMed: 1640258]
30. Merchant K, Martorell R. Frequent reproductive cycling: does it lead to nutritional depletion of mothers? Prog Food Nutr Sci 1988;12(4):339–369. [PubMed: 3077180]
31. Miller J, Rodriquez G, Pebley A. Lactation, seasonality, and mother's postpartum weight change in Bangladesh: an analysis of maternal depletion. American Journal of Human Biology 1992;6:511–524.
32. Nduati R, Richardson BA, John G, et al. Effect of breastfeeding on mortality among HIV-1 infected women: a randomised trial. Lancet 2001;357(9269):1651–1655. [PubMed: 11425369]
33. Macallan DC. Wasting in HIV infection and AIDS. J Nutr 1999;129(1S Suppl):238S–242S. [PubMed: 9915906]
34. Thior I, Lockman S, Smeaton LM, et al. Breastfeeding plus infant zidovudine prophylaxis for 6 months vs formula feeding plus infant zidovudine for 1 month to reduce mother-to-child HIV transmission in Botswana: a randomized trial: the Mashi Study. JAMA 2006;296(7):794–805. [PubMed: 16905785]
35. Wilfert CM, Fowler MG. Balancing maternal and infant benefits and the consequences of breast-feeding in the developing world during the era of HIV infection. J Infect Dis 2007;195(2):165–167. [PubMed: 17191159]

36. Report of a technical consultation. WHO; 2007 [Accessed 21st August 2008]. HIV and Infant Feeding: New evidence and programmatic experience. http://whqlibdoc.who.int/publications/2007/9789241595971_eng.pdf
37. Moses A, Zimba C, Kamanga E, et al. Prevention of mother-to-child transmission: program changes and the effect on uptake of the HIVNET 012 regimen in Malawi. AIDS 2008;22(1):83–87. [PubMed: 18090395]
38. Fawzi WW, Msamanga GI, Hunter D, et al. Randomized trial of vitamin supplements in relation to transmission of HIV-1 through breastfeeding and early child mortality. AIDS 2002;16(14):1935–1944. [PubMed: 12351954]
39. Shetty AK, Coovadia HM, Mirochnick MM, et al. Safety and trough concentrations of nevirapine prophylaxis given daily, twice weekly, or weekly in breast-feeding infants from birth to 6 months. J Acquir Immune Defic Syndr 2003;34(5):482–490. [PubMed: 14657758]
40. Taha TE, Kumwenda NI, Hoover DR, et al. The impact of breastfeeding on the health of HIV-positive mothers and their children in sub-Saharan Africa. Bull World Health Organ 2006;84(7):546–554. [PubMed: 16878228]
41. Briend A, Lacsala R, Prudhon C, Mounier B, Grellety Y, Golden MH. Ready-to-use therapeutic food for treatment of marasmus. Lancet 1999;353(9166):1767–1768. [PubMed: 10347999]
42. Briend A. Highly nutrient-dense spreads: a new approach to delivering multiple micronutrients to high-risk groups. Br J Nutr 2001;85 (Suppl 2):S175–S179. [PubMed: 11509107]
43. Ciliberto MA, Sandige H, Ndekha MJ, et al. Comparison of home-based therapy with ready-to-use therapeutic food with standard therapy in the treatment of malnourished Malawian children: a controlled, clinical effectiveness trial. Am J Clin Nutr 2005;81(4):864–870. [PubMed: 15817865]
44. Collins S, Sadler K. Outpatient care for severely malnourished children in emergency relief programmes: a retrospective cohort study. Lancet 2002;360(9348):1824–1830. [PubMed: 12480359]
45. Linneman Z, Matilsky D, Ndekha M, Manary MJ, Maleta K, Manary MJ. A large-scale operational study of home-based therapy with ready-to-use therapeutic food in childhood malnutrition in Malawi. Matern Child Nutr 2007;3(3):206–215. [PubMed: 17539889]
46. Breastfeeding and HIV International Transmission Study Group. Mortality among HIV-1-infected women according to children's feeding modality: an individual patient data meta-analysis. J Acquir Immune Defic Syndr 2005;39(4):430–438. [PubMed: 16010166]
47. Kourtis AP, Jamieson DJ, de Vincenzi I, et al. Prevention of human immunodeficiency virus-1 transmission to the infant through breastfeeding: new developments. Am J Obstet Gynecol 2007;197 (3 Suppl):S113–S122. [PubMed: 17825642]
48. Kourtis AP, Lee FK, Abrams EJ, Jamieson DJ, Bulterys M. Mother-to-child transmission of HIV-1: timing and implications for prevention. Lancet Infect Dis 2006;6(11):726–732. [PubMed: 17067921]
49. Bentley ME, Corneli AL, Piwoz E, et al. Perceptions of the role of maternal nutrition in HIV-positive breast-feeding women in Malawi. J Nutr 2005;135(4):945–949. [PubMed: 15795467]
50. Corneli AL, Piwoz EG, Bentley ME, et al. Involving communities in the design of clinical trial protocols: the BAN Study in Lilongwe, Malawi. Contemp Clin Trials 2007;28(1):59–67. [PubMed: 17000137]
51. UNDP Human development Reports 2007-8. [Accessed 21st August 2008]. http://hdrstats.undp.org/countries/data_sheets/cty_ds_MWI.html
52. Hitti J, Frenkel LM, Stek AM, et al. Maternal toxicity with continuous nevirapine in pregnancy: results from PACTG 1022. J Acquir Immune Defic Syndr 2004;36(3):772–76. [PubMed: 15213559]
53. Kourtis AP, Bramson B, van der HC, et al. Low absolute neutrophil counts in african infants. J Int Assoc Physicians AIDS Care (Chic Ill) 2005;4(3):73–76. [PubMed: 16867975]
54. Wells J, Shetty AK, Stranix L, et al. Range of normal neutrophil counts in healthy zimbabwean infants: implications for monitoring antiretroviral drug toxicity. J Acquir Immune Defic Syndr 2006;42(4): 460–463. [PubMed: 16810112]
55. Mackie NE, Fidler S, Tamm N, et al. Clinical implications of stopping nevirapine-based antiretroviral therapy: relative pharmacokinetics and avoidance of drug resistance. HIV Med 2004;5(3):180–184. [PubMed: 15139985]
56. FDA Public Health Advisory for Nevirapine (Viramune). [Accessed 21st August 2008]. http://www.fda.gov/CDER/drug/advisory/nevirapine.htm

57. WHO; 2006 [Accessed 21st August 2008]. WHO Guidelines on co-trimoxazole prophylaxis for HIV-related infections among children, adolescents and adults: recomendations for a public health approach. http://www.who.int/hiv/pub/guidelines/ctxguidelines.pdf
58. Beck EJ, Vitoria M, Mandalia S, Crowley S, Gilks CF, Souteyrand Y. National adult antiretroviral therapy guidelines in resource-limited countries: concordance with 2003 WHO guidelines? AIDS 2006;20(11):1497–1502. [PubMed: 16847404]
59. Hosseinipour MC, Corbett AH, Kanyama C, et al. Pharmacokinetic comparison of generic and trade formulations of lamivudine, stavudine and nevirapine in HIV-infected Malawian adults. AIDS 2007;21(1):59–64. [PubMed: 17148968]
60. PETRA Study Team. Efficacy of three short-course regimens of zidovudine and lamivudine in preventing early and late transmission of HIV-1 from mother to child in Tanzania, South Africa, and Uganda (Petra study): a randomised, double-blind, placebo-controlled trial. Lancet 2002;359(9313): 1178–1186. [PubMed: 11955535]
61. CROI; 2008 [Accessed 21st August 2008]. Extended infant post-exposure prophylaxis with antiretroviral drugs significantly reduces postnatal HIV transmission: The PEPI-Malawi Study. http://www.retroconference.org/AbstractSearch/Default.aspx?Conf=17
62. Dabis F, Bequet L, Ekouevi D, et al. Field efficacy of zidovidine, lamivudine and single dose nevirapine to prevent peripartum HIV transmission. AIDS 2005;19(3):308–318.
63. Guay LA, Musoke P, Fleming T, et al. Intrapartum and neonatal single-dose nevirapine compared with zidovudine for prevention of mother-to-child transmission of HIV-1 in Kampala, Uganda: HIVNET 012 randomised trial. Lancet 1999;354(9181):795–802. [PubMed: 10485720]
64. Thea DM, Vwalika C, Kasonde P, et al. Issues in the design of a clinical trial with a behavioral intervention--the Zambia exclusive breast-feeding study. Control Clin Trials 2004;25(4):353–365. [PubMed: 15296810]
65. CROI; 2008 [Accessed 21st August 2008]. Prevention of mother-to-child transmission of HIV-1 among breastfeeding mothers using HAART: The Kisumu Breastfeeding Study, Kisumu, Kenya 2003–2007. http://www.retroconference.org/2008/Abstracts/33397.htm
66. Moodley D for the SAINT Investigators Team. The SAINT trial: nevirapine versus zidovudine + lamivudine in prevention of peripartum HIV transmission. Abstract presented at: XIII International AIDS Conference; Durban, South Africa. July 9–14, 2000; Abstract LbOr2
67. Committee on Reviewing the HIVNET 012 Perinatal Prevention Study. Review of the HIVNET 012 Perinatal HIV Prevention Study. Institute of Medicine 2005.
68. Montaner L, McIntyre J, van der Horst CM. Change in US research policies and directives are needed to remove barriers on HIV-1 operational research programs in developing countries: a case report of two NIH clinical trials in South Africa. Harvard Health Policy Review 2005;6:114–123.
69. Newell ML, Coovadia H, Cortina-Borja M, Rollins N, Gaillard P, Dabis F. Mortality of infected and uninfected infants born to HIV-infected mothers in Africa: a pooled analysis. Lancet 2004;364(9441): 1236–1243. [PubMed: 15464184]
70. Country Statistics. Infant mortality rate (year 2005). UNICEF; 2008 [Accessed 21st August 2008]. http://www.unicef.org/infobycountry/malawi_statistics.html
71. Estimates developed by WHO, UNICEF, and UNFPA. WHO; 2007 [Accessed 21st August 2008]. Maternal mortality in 2005. http://www.who.int/reproductive-health/publications/maternal_mortality_2005/index.html
72. UN Common Database. Malawi life expectancy for females (estimate for 2005). United Nations; 2008 [Accessed 21st August 2008]. http://unstats.un.org/unsd/cdb/cdb_advanced_data_extract_yr.asp?HSrID=13630&HCrID=454
73. Muula AS, Panulo B Jr, Maseko FC. The financial losses from the migration of nurses from Malawi. BMC Nurs 2006;5:9. [PubMed: 17081302]

Table 1

Factorial design treatment arms

	No ARV	Infant ARV	Maternal HAART
No nutritional supplement	403 No supplement No ARV	403 No supplement Infant Daily NVP	403 No supplement Maternal HAART
Nutritional supplement	403 Supplement No ARV	403 Supplement Infant Daily NVP	403 Supplement Maternal HAART

Table 2

Composition of maternal nutritional supplement

Component	Provides
Energy	700 kcal
Protein	20 g
Iron	15 mg
Zinc	19 mg
Phosphorus	1200 mg
Selenium	75 μg
B_1 (thiamin)	1.6 mg
B_2 (riboflavin)	1.8 mg
B_3 (niacin)	20 mg equiv
B_6 (pyridoxine)	2.2 mg
B_{12} (cyanocobalamine)	2.6 μg
C (ascorbic acid)	100 mg
E (α-tocopherol)	12 mg
Folic acid	300 μg
Iodine	200 μg

Table 3

Infant dosing regimen of nevirapine

Age	Dose	Rounded-off dose
0–7 days	0.6 mL (6 mg) once daily	1 mL
7–14 days	0.6 mL (6 mg) once daily	1 mL
3–4 weeks	1.5 mL (15 mg) once daily	2 mL
5–6 weeks	1.8 mL (18 mg) once daily	2 mL
7–8 weeks	2.0 mL (20 mg) once daily	2 mL
9–12 weeks	2.2 mL (22 mg) once daily	2 mL
13–18 weeks	2.4 mL (24 mg) once daily	2 mL
19–20 weeks	2.6 mL (26 mg) once daily	3 mL
21–28 weeks	2.8 mL (28 mg) once daily	3 mL

Table 4

Protocol Amendments

Protocol	Revisions
April 2003	Protocol submitted to three IRBs (UNC, CDC, and Malawi) for initial review.
Nov 2003	As three primary approvals (by UNC, CDC, Malawi) on different versions of protocol, amendment #1 submitted to all IRBs to reconcile minor change
June 2004	Splitting consent process into screening and enrolment and increasing monitoring for hepatic toxicity
Sept 2004	Modifying dose of 3TC of infants switched from NVP. Modification of neutrophil cutpoints in infants
Dec 2004	Addition of 7 day tail of CBV at 28 wks for those assigned NVP and follow-up of HIV-infected infants beyond 2 weeks
Dec 2004	Informing IRBs of temporary change from NVP to second-line NFV for safety reasons.
June 2005	Changing study drug NVP to Lopinavir/ritonavir and SAE Reporting modifications
Nov 2005	Increasing reimbursement to equivalent of $4 in Malawi currency.
Mar 2006	Update NIH DAIDS Toxicity Table. Drop mothers' dietary recall at 28 weeks to ease patient flow.
May 2006	Change in CD4 count eligibility: enroll only if >250 to meet new Malawi criteria for treatment. Drop mothers' dietary recall at 32 and 48 weeks decrease time in clinic. Accept CD4 count from PMTCT program antenatally. Eliminate antenatal anthropometric measurements after baseline to simplify procedures.
May 2007	LPV/r: Kaletra capsules changed to Aluvia tablets. Decrease breastfeeding questionnaires, breast physical exam forms, and all dietary recalls. Stop storing cell pellets, PBMCs, PK samples. Decrease amount of breast milk stored. Mucosal substudy eliminated, reimbursement increased to Malawi equivalent of $8 for intensive substudies. Addition of substudy – hepatitis study based on stored specimens.
July 2007	Amend consents to obtain permission to contact after end of study (at 48 weeks) for potential future studies.

Table 5

NIH Division of AIDS previous and revised toxicity tables for definition of infant neutropenia and BAN modification

DAIDS Toxicity Table				
Cutpoints of infant neutropenia (cells/μL)				
	Day 1	**Day 2–7**	**Day 8–56**	**Day > 57**
Grade 1	5000–7000	1750–2500	1200–1800	750–1200
Grade 2	3000–5000	1250–1750	900–1200	400–750
Grade 3	1500–3000	750–1,250	500– 900	250–400
Grade 4	< 1,500	< 750	< 500	<250
Revised DAIDS Toxicity Table (*December 2004*)				
Cutpoints for infant neutropenia (cells/μL)				
	Day 1	Day 2–7	Day 8–56	Day > 57
Grade 1	4000–5000	1250–1500	1000–1300	1000–1300
Grade 2	3000–4000	1000–1250	750–1000	750–1000
Grade 3	1500–3000	750–1,000	500– 750	500–750
Grade 4	< 1,500	< 750	< 500	<500
BAN Modification of Toxicity Table				
Cutpoints for infant neutropenia (cells/μL)				
	Day 1	Day 2–7	Day 8–56	Day > 57
Grade 1	750–1200	750–1200	750–1200	750–1200
Grade 2	400–750	400–750	400–750	400–750
Grade 3	250–400	250–400	250–400	250–400
Grade 4	< 250	< 250	< 250	<250